A Synopsis of thesis entitled

SCREENING AND CHARACTERIZATION OF MEDICINAL PLANT EXTRACTS AND MOLECULES AS THE SOURCE OF NEW ANTI-SNAKE VENOM DRUGS

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By

Mr. Nagargoje Digambar Ramrao
M. Sc., M. Phil.
Research Scholar

Under Supervision of

Dr. Suresh M Telang
Research Supervisor
DEPT. OF BOTANY
YESHWANT MAHAVIDHYALAYA (SR.) COLLEGE,
NANDED – 431 602

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Introduction

Snake bite is an important and serious medico legal problem in many parts of the world, especially in South Asian countries. Estimates of global mortality from snake bite have been reported to range from 50,000 per year (Swaroop and Grab, 1954) to 1,00,000 per year (Chippaux, 1998). As recently reviewed, globally, venomous snakebites is estimated to affect more than 4,21,000 humans per year, with 20,000 fatalities. If we take into account the non-reported accidents, these data may be as high as 18,41,000 snake envenomingsand 94,000 deaths (Kasturirante et al., 2008). Venomous snakebites continue to be a major medical concern in India, as it has been for at least last 100 years. In the 1950s, a World Health Organization (WHO) survey estimated the number of bites at 2,00,000 in India alone with a mortality of 15,000 annually (Swaroop and Grab, 1954). More recent in the report WHO estimated the annual mortality due to snake bite in India is approximately 50,000 (Warell, 1999). Snakes are taxonomically carnivorous vertebrates of the class Reptilia, order Squamata, sub-order Serpentes and families : Colubridae, Boidae, Elapidae, Pythonidae, Viperidae that characteristically kill their prey with constriction rather than envenomation, though venomous snakes are prevalent in most continents of plant earth with the exception of Antarctica, Ireland, New Zealand and many small islands of the Atlantic and Central Pacific region. Most bites are due to circumstantial stepping on the snakes by unprotected or barefooted victims, snake ecology, while a few others are initiated by malevolent victims and alcohol intoxications. Over 3,500 species of snakes have been taxonomically reported. Approximately 600 (15-17%) of these are venomous and 200 (5.7%) are regarded as medically significant.

The essence of the fascination for and fear of snakes has lain in their venom. Snake venom is a complex mixture of proteins and other organic and inorganic materials, which have a diverse array of actions both on prey and human victims. The major components are biologically highly active proteins whose primary function is to kill or immobilize the prey and also to assist in the digestion of that prey. The biologically active components present in snake venoms can be either enzymes or non enzymatic proteins. Among snake venom enzyme, phospholipase A_2 (PLA_2)is widely distributed and is found in almost all snake venoms (Rosenberg, 1979; Fletcher et al., 1979). Venom PLA_2s show wide variety of toxicity and induce several pathological symptoms (Karlsson, 1979; Howard and Gunderson, 1980; Mebs, 1983; Kini and Evans, 1989)

in addition to their contribution along with the various hydrolytic enzymes, in the digestion of prey (Tu, 1977).

Among phospholipases, phospholipase A_2(PLA_2) has been studied extensively. PLA_2 purified from different sources have been reviewed well in early reviews of Slotta (1960) and Hanahan (1971). Several reviews have prepared specifically on extracellular PLA_2 (Verheij et al., 1981; Rosenberg, 1986). Snake venom PLA_2 enzymes show a wide variety of toxicities and induce several pathological symptoms, in addition to their contribution in digestion of the prey. They induce neurotoxic(Fraenkel-Conrat, 1983), myotoxic (Gopalakrishnakone et al., 1984; Kasturi and Gowda, 1989), cardiotoxic (Fletcher et al., 1981; Chang et al., 1983), edema inducing (Jayanthi et al., 1989; Bhat and Gowda, 1989), anticoagulant (Kini and Evans, 1987; Kasturi and Gowda, 1990a), hemolytic (Shukla and Hanahan, 1982, Vishwanath et al., 1987a), convulsant and hypotensive (Fltcher et al., 1980; Huang, 1984) effects in victims.

Review of Literature and Development in the subject (Previous work done in the relevant area):

Still today snake bite is a common acute medical emergency faced by rural population in tropical and subtropical countries with heavy rainfall and humid climate (Banerjee, 1978; Bhat, 1974). There are no accurate records available to determine the exact epidemiological or even snakebite cases in Maharashtra. One of the widely accepted concepts in Indian snake venom discussion and research is the notion of the so-called "Big 4 snakes of medical importance" which includes the Russell's viper (*Viperarusselli*), the saw scaled viper (*Echiscarinatus*), the Indian or spectacled cobra (*Najanaja*) and the common krait (*Bungarus caeruleus*). These four snakes believed to cause the vast majority, if not all, fatalities to snakebites (Ian and Robert, 2007).Recently in a study of snakebite envenoming in rural Maharashtra, Bawaskar et al., (2008) reported that 182 cases of snakebite were admitted during 12 months at 5 hospitals situated in five different districts of rural Maharashtra. Out of these 55 (30.2%), 38 (20.8%), 48 (26.3%), 41 (22.5%) cases were bitten by *Echiscarinatus,* Russell's viper, krait and cobra respectively.

Anti snake venom (ASV) is a specific antidote to snake venom actions. Surprisingly enough still it is used in empirical way to manage the victims of poisonous snake bite case. Snake venom antiserum development and standardization is expensive and requires ideal storage conditions; storage facility for antiserum may be lacking in the usually rural snake endemic area of Marathwada. In Maharashtra, polyvalent ASV is available which contain antibody against

cobra, Russell's viper, common krait and saw-scaled viper. It is usually not possible to accurately identify the species of snake involved in cases of snake bite and since different families of snake produce venoms with different pharmacological activities, clinical treatment often involves use of a polyvalent antivenin. The exact dose of venom injected at the time of bite by the snake is not known; similarly the amount of ASV required to neutralize the circulating venom cannot be detected clinically. Hence, dose of ASV administered varies from doctor to doctor.Many times anti-venom is not available in local medical center or is not accessible to the villagers living in remote areas.At private hospital treatment of poisonous snakebite is not affordable. Many times medical officers are absent and not available at head quarters during night hours. These are some problems associated with modern ASV therapy in rural areas.

Today antivenom therapy is an only therapy to treat snakebite victims. There are two primary risks associated with antivenom therapy viz., acute anaphylaxis or anaphylactic reactions (mild to severe) which occur within one hour of antivenom administration (Singh et al., 2001). Antiserum sometimes does not provide enough protection against snake venom-induced haemorrhage, necrosis, nephrotoxicity and often produced hypersensitive reactions (Corrigan et al., 1987). Although it is well known that antiserum is invariably unavailable in remote rural Marathwada, the role of medicinal plants remains largely unnoticed and neglected. The use of plants as alternative for treatment of poisonous bites is important in remote areas where there is no accessibility to hospitals and storage facility for antivenoms. Therefore, it is important to explore their materia medica for alternative venom antidotes that could accompany or substitute conventional antivenoms. Plants are popular alternatives for the treatment of poisonous snakebites (Nuno et al., 1994; Selvanayagam et al., 1995). Moreover, some of these traditional methods have a scientific basis (Abubakar et al., 2000; Alam and Gomes, 2003).

The World Health Organization (WHO) defines traditional medicine to include a diversity of health practices, approaches, knowledge, and beliefs incorporating plant, animal, and/or mineral-based medicines; spiritual therapies; manual techniques; and exercises, applied singly or in combination to maintain well-being, as well as to treat, diagnose, or prevent illness. (WHO, 2001).Plant forms the main ingredients of medicine in traditional system of healing and has been the source of inspiration for several major pharmaceutical drugs (MacDonald, 2009).

In almost any part of the world, where venomous snakes occur, numerous plant species are used to treat snakebite (Martz, 1992). In India also many medicinal plants are recommended

for the treatment of snakebite (Chopra et al., 1956). India has long ancient tradition for treating various ailments with the use of medicinal plants. Plants are used either single or in combination, as antidotes for snake envenomation by rural populations in India and in many parts of the world. Plants are reputed to neutralize the action of snake venom, with a plethora of plants claimed to be antidotes for snakebites in traditional folk medicine (Kirtikar and Basu, 1975).

The use of plants against the effects of snake bite has long been recognized (Bocquillon-Limousin, 1891) even in modern times but only for the last 20 years has it merited closer scientific attention (Selvanayagam et al, 1995). Several reviews about the subject have by now been published (Mors, 1991; Martz, 1992; Houghton and Osibogun, 1993; Selvanayagam et al, 1994, 1995).In almost any part of the world, where venomous snakes occur, numerous plant species are used to treat snakebite (Martz, 1992). Japanese workers reported about the detoxifying action of persimmon tannin (from the unripe fruits of *Diospyros kaki*) as an acknowledged medicine against snake venom envenomation, in Japan (Okonogi et al., 1979). In the first scientific work on the subject, the extract of the *Curcuma longa* plant was shown to inactivate almost completely the neurotoxin of the cobra, *Najanajasiamensis* (Cherdchu and Karlsson, 1983). Attempts are continuously being made to isolate active principles from plants to neutralize venom toxicity. Various workers have reported the neutralizing effect of tannic acid against snake venom (Homma et al., 1965; Okonogi and Hattori, 1978). Okonogi et al., (1970) devised a kit containing a 5% tannic acid solution to be used as an emergency treatment for poisonous snake bites.

It has been observed that a striking parallism exists between the capabilities of plants and their chemical compounds in neutralizing the action of snake venom toxicity (Mors et al., 2000). With these inhibitors, mechanism of action of several venom toxins/enzymes has been elucided (Jayraman et al., 1999; Mors et al., 2000). Dhananjaya et al., (2006) in their studies on anticoagulant effect of *Najanaja* venom demonstrated that vanillic acid inhibited anticoagulant activity. Apart from better characterized glycoproteins from snakes and mammals, which exhibit inhibitory properties against PLA_2 from snakes and their toxic properties, special mention is made of a newly described glycoprotein isolated from *Withaniasomnifera* (Aswagandha), a medicinal plants reputed to have multiple pharmacological effects. Several plant constituents like flavonoids, quinonoid, xanthenes, polyphenols and terpenoids possessed protein binding and enzyme inhibiting properties, which also inhibit snake venom PLA2 of viper and cobra

venom(Selvanayagam et al., 1996). It is remarkable that the 4-O-methy ether of the first was identified as a snake venom neutralizing factor in the Indian antisnake venom plant *Hemidesmus indicus* (Alam et al., 1994). In all above mentioned cases, the selection of medicinal plants for antivenom study was primarily depending on its traditional use for treatment of snake bite.

The exploration of traditional knowledge regarding the perception and practice of medicinal plants prescribed in indigenous traditional literature provides excellent opportunity to familiarize the explorer with the plant wealth and natural remedies for treating poisonous snake bites. Academically it is an excellent opportunity to establish the scientific basis for the usage of traditional antivenom medicinal plants. Such attempts may suggest alternative medicines to the local community of this region.

Taking into consideration the above facts and need to explore the plant wealth of Indian traditional literature and immediate need to find alternatives for the treatment of poisonous snakebite; all these circumstances prompted investigator to design this study. The proposed work will focus on the investigation of phytotherapy for treating poisonous snake bites, customized and documented in Indian traditional literature. And to evaluate systematically selected medicinal plants against *Najanaja* venom in search of novel antivenom agents with minimum or no cytotoxicity to humans.

Objectives of Research/ Proposed Hypothesis:

The objectives of the proposed research work are as follows:

- To Investigate traditional phytotherapy customized and documented in Indian Traditional literature for treating poisonous snakebite cases and record Photographic documentation, collection, identification and authentication of medicinal plants prescribed in Indian traditional literature for the treatment of snake bite.
- To analyse plant with different solvents for partitioning the plant ingredients and biochemical characterization of selected medicinal plants
- To evaluatephytochemical fractions of selected plant samples against *Naja naja* venom for potential antivenom target ssuch as Phospholipase A_2 inhibition activity, anti-proteolytic activity, anticoagulant activity and antihemolytic activity.

- To prepare HPTLC fingerprints of selected medicinal plants for *in vitro*evaluation of cytotoxicity of selected medicinal plants with antivenom activity.

Methodology to be adopted:

1) Field Survey and documentation of traditional knowledge:

Field surveys were undertaken to gather data on the traditional use of medicinal plant species across various traditional healers in Nanded district. Traditional healers, called '*Vaidya'* or '*Janata'* from different indigenous groups were targeted for documentation of the uses of medicinal plants. Regular visit to local traditional healers is arranged for observation of their treatment method. Local traditional healers having practical knowledge of plants in treating the snakebite were interviewed in four villages of the district. The information was gathered from five traditional vaidyas. Since only these vaidyas are well-known in this region for treating snakebite patients over several years and on the basis of the knowledge they have and beliefs of local people, the traditional healers were selected for interviews.

The ethnomedicinal data were collected through general conversations with the vaidyas. The questionnaires were used to obtain information on medicinal plants with their local names, parts used, mode of preparation and administration. No monetary compensation has been given to the traditional healers for providing the medicinal information.

2) Plant collection and identification:

The standard method was followed with regard to collection of plant material, drying, mounting, preparation and preservation of plant specimens (Jain and Rao, 1976). The identification and nomenclature of the plants were based on the Flora of Marathwada (Naik, 1998). The selected medicinal plants are *Argemone Mexicana* L., *Amorphophallus sylvaticus* (Roxb.) Kunth, *Calotropis gigantea* L., *Caesalpinia bonduc* (L.) Roxb., *Dioscorea pentaphylla* L., *Eclipta alba* L., *Solanum nigrum* L. subsp. *Nigrum* and *Momordica dioica* Roxb., The voucher specimens with catalog number were deposited in the herbarium of Department of Botany, Yeshwant Mahavidyalaya (Sr.) College, Nanded.

3) Phytochemical screening of selected medicinal plants:

The qualitative phytochemical tests were carried out to identify the active constituent as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The quantitative phytochemical tests were performed for tannin (Van-Burden and Robinson, 1981), saponin

(Obadoni and Ochuko, 2001), flavonoid (Bohm and Kocipai-Abyazan, 1974), alkaloids (Harborne, 1973) and phenols (Lowry et al., 1951).

Importance of study

International Status:

Worldwide about 30,000 to 40,000 people die annually of snake bites. Of these, about 25,000 people die in India, mostly in rural areas, about 10,000 people in United States and rest of in other countries. Under the Wild Life Protection Act, 1972, all snakes are protected.. Snake venom is badly needed to produce anti venom required to treat potentially fatal snakebites (Dravidamani et al., 2008).In many traditional literatures all over the World, many phytochemicals have been identified which are used to neutralize the effects of snake venoms. The way of management of snake bites through herbals are by treating with single herbal drugs or in combination applications.. With reflection to that area, an attempt is being made to collect available information about some medicinal plants advancement against snakebites.Recent scientific investigations have confirmed the efficacy of many of these preparations, some of which are remarkably effective. Traditional Indian medicinal drugs viz. *Argomone maxicana,Calotropis gigantia,Tamirandus indica,Thea sinensis,Aristolochia spps, Excoecaria agallocha, Gloriosa superba,Acalypa indica, Nerium oleander,Eclipta alba, Sarsaparilla hemidesmus etc* and many others are effectively used for snake bites but still lot of clinical and pre-clinical research needs for the evidence. The herbs which appear most effective as per the symptoms of snakebites are relatively non-toxic and have substantial documented efficacy, among them some herbs *Aristolochia species, Cissus assamica, Echinacea species, Guiera senegalensis, Hemidesmus indicus, Parkia biglobosa, Securidaca longipedunculata, Tamarindus indica, Trianosperma tayuya, Thea sinensis, Withania somnifera,* are enlisted with proper informations for future research Snakebite is one of the important medical problems that affect the public health due to their high mortality. Most of the snake venoms produce intense lethal effects, which could lead permanent disability or in often death to the victims. The accessible specific treatment was using the antivenom , whose efficiency is reduced against these lethal actions but it has a serious side effects. In this circumstance, this study aimed to provide an updated overview of herbal plants used popularly as antiophidic agents and discuss the main species with pharmacological studies supporting the uses, with prominence on plants inhibiting the lethal effects of snake envenomation amongst the rural tribal peoples of India,with special

reference to Indian traditional literature. There are several studies which shows the acceptance of use of phytochemicals against snakebites worldwide. In recent years, many studies have been published to giving pharmacological confirmation of benefits of several plant species against local effects observed by a snake venoms, including inhibitory potential against, phospholipase, proteolytic, hemorrhagic, myotoxic,neurotoxic and inflamatory activities. The present study was designed to formulate an attempt to bring together information on medicinal plants that are grown and used for snakebite treatment in traditional literature of India. From a literature sources, data have been,collected, compiled with potential of the plants, family, parts used, etc., as mentioned in the available literature and information. Hippocrates also stated that "Nature cures, not the physician." Plants are rich sources of medicinal Traditional herbalists function closer to the people, taking improvement of the biodiversity of plant species in such areas to cure various diseases and ailments (Houghton & Osibogun, 1993).

National Status:

India is the seventh largest country among many which has a wide variety of medicinal plants growing under various geographical and ecological conditions. Over 1500 species out of about 15,000 privileged plants species in India have been reported to have medicinal uses (Kosalge & Fursule, 2009). Moreover, there are many more local or tribal dialects spoken by Indians along with regional languages. India is home to different ethnic groups, having diverse cultures, religious rites, and food traditions that separate them from each other. These people also have a healthy awareness of traditional medicine, especially herbal and folk medicine for treatment in snakebites (Harsha et al., 2002; Parinitha et al., 2005). Many Indian medicinal plants or plant materials are recommended for the treatment of snakebite and some are tested, but so far no systematic analysis has been done (Harsha et al., 2002; Parinitha et al., 2005; Samy et al., 2008; Bhandary, 1996). In India, there is a group of people known as "Vaidya" (a doctor of herbs, compounder of medicinal preparations such as Churna, Pills, Syrup, Asava, Aristha, Taila), and elderly persons with folk knowledge about the uses of various house hold spices and herbs. Their native beliefs, skills, and cultural practices concerned with people's health are useful in curing various diseases. Some elderly women with expertise in delivering babies have proficient knowledge about folk medicines for facing day to day health problems. As per Indian beliefs, Mother Nature nurtures us.

Significance of the study

Snake bite by venomous snakes is a cause of concern in many countries of the world where various species of venomous snakes are still present in large numbers and are in frequent contact with people like rural and remote area inhabitants, where anti-venom and modern treatment for snake bites are absent. Such people are still dependent on traditional medicinal practitioners, whose mainstay of treatment is phytotherapy or treatment with plants. Despite their apparent backwardness, surprisingly scientists are discovering that many of these plants or rather phytochemicals present in these plants can neutralize venom of a number of the most venomous snakes of the world. In this review, we describe and analyze anti-snake venom plants,. These ee plants are used in the Indian traditional literatures for treatment of snake bites and which have potential for discovery of drugs against snake bites.

Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved

First year (2021 -2022):

1. Survey of traditional phytotherapy customized and documented in Indian Traditional literature for treating poisonous snakebite cases.
2. RecordingPhotographic documentation, collection, identification and authentication of medicinal plants prescribed in Indian traditional literature for the treatment of snake bite.

Second year (2022 -2023):

1. Analysis of plant with different solvents for partitioning the plant ingredients.
2. Biochemical characterization of selected medicinal plants for the treatment of snake bite.
3. Evaluation of phytochemical fractions of selected plant samples against *Naja naja* venom for potential antivenom targets

Third year (2023 -2024):

1. Screening of antivenom targets such as Phospholipase A_2 inhibition activity, anti-proteolytic activity, anticoagulant activity and antihemolytic activity.

2. To prepare HPTLC fingerprints of selected medicinal plants for in vitro evaluation of cytotoxicity of selected medicinal plants with antivenom activity
3. Analysis and Compilation of work

Expected outcome

At present, serum antivenom immunotherapy is the only treatment reported for snake envenomation . Antisera are either derived from horse serum after injecting the horse with sublethal doses of snake venom or sheep serum. Though antivenin is lifesaving, it carries some side effects such as development of immediate or delayed hypersensitivity (serum sickness). For efficacy, the antidote should be administered within 4-6 hours of the snakebite. The side effects of intravenous antivenoms are thought to be the aftermath of the action of non-immunoglobulin proteins present in higher concentrations in the antivenom . Worse still, there is unavailability of snake antivenom serum in rural India that even in the presence of money, it may not be readily available for purchase. In this perspective, several attempts have been made to develop snake venom antagonists from other sources including plants, dogs, rabbits, and avian eggs.

References:

- Swaroop S, Grab B. (1954): Snake bite mortality in the world. Bull WHO. 10, 35-75.
- Chippaux JP. (1998): Snake bites: appraisal of the global situation. Bull. WHO. 76, 515-524.
- Kasturirante A, Wickremasinghe A. R., Silva N., Gunawardena N. K. and Pathmeswaran A. (2008): The Global burden of snakebite: A literature analysis and modeling based on regional estimates of envenoming and deaths. PLoS Medicine, 5: 1591-1604.
- Warrell D. A. (1999): The clinical management of snakebites in the Southeast Asian region. Southeast Asian Journal of Tropical Medicines and Public Health. 30, 1-84.
- Fletcher J. E., Elliott W. B., Ishay J. and Rosenberg P. (1979): Affidavit Phospholipase A and B activities of reptile and hymenoptera venoms. Toxicon, 17: 591.
- Karlsson E. (1979): Chemistry of protein toxins in snake venoms. In: Snake venoms. Handbook of Experimental Pharmacology. (Lee C. Y. Ed.), Vol. 52, pp. 159. Berlin: Springer-Verlag.

- Howard B. D. and Gundersen C. B. Jr. (1980): Effects and mechanisms of polypeptide neurotoxins that act presynaptically. Ann. Rev. Pharmac. Toxicol., 20: 307.
- Kini R. M. and Evans H. J. (1989): Affidavit model to explain the pharmacological effects of snake venom Phospholipase A_2. Toxicon, 27: 613.
- Tu A. T. (Ed.) (1977): Venoms: Chemistry and Molecular Biology. New York: John Wiley.
- Slotta K. H. (1960): The Enzymes. 2nd Edn. Vol. IV, pp 551.
- Hanahan D. J. (1971): The Enzymes, 3rd Edn. Vol. V, pp 71.
- Verheij H. M., Slotboom A. F. and De Haas G. H. (1981): Structure and function of Phospholipase A_2. Rev. Physiol. Biochem. Pharmacol., 91: 91.
- Rosenberg P. (1986): The relationship between enzymatic activity and pharmacological properties of Phospholipases in natural poisons. In: Natural Toxins: Animal, Plants and Microbial. Pp, 129. (Harris J. B. Ed.) Oxford: Oxford Uni. Press.
- Fraenkel – Conrat H. (1982-83): Snake venom neurotoxin related to Phospholipase A_2. J. Toxicol.- Toxin Rev. 1: 205.
- Gopalkrishnakone P., Dempster D. W., Hawgood B. J. and Elder H. Y. (1984): Cellular and mitochondrial changes induced in the structure of murine muscle by crotoxin, a neurotoxic Phospholipase A_2 complex. Toxicon, 22: 85.
- Kasturi S. and Gowda T. V. (1989): Purification and characterization of major Phospholipase A_2 from Russell's viper (*Viperarusselli*) venom. Toxicon, 27: 229.
- Fletcher J. E., Rapuano B. E., Condrea E., Yang C. C. and Rosenberg P. (1981): Relationship between catalysis and toxicological properties of three Phospholipase A_2 from elapid snake venom. Toxicol. Appl. Pharmac., 59: 375.
- Chang W. C., Lee M. L. and Lo T. B. (1983): Phospholipase A_2 activity of long-chain cardiotoxins in the venom of branded krait (*Bungarus fasciatus*). Toxicon, 21: 163.
- Jayanthi G. P., Kasturi S. and Gowda T. V. (1989): Dissociation of catalytic activity and neurotoxicity of a basic Phospholipase A_2 from Russell's viper (*Viperarusselli*) venom. Toxicon, 27: 875.
- Bhat M. K. and Gowda T. V. (1989): Purification and characterization of a Phospholipase A_2 from Indian cobra (*Najanajanaja*) venom. Toxicon, 27: 861.
- Kini R. M. and Evans H. J. (1987): Structure-function relationship of Phospholipases: the anticoagulant region of Phospholipase A_2. J. Biol. Chem. 262 (14): 402.

- Shukla S. D. and Hanahan D. J. (1982): Identification of domains of phosphatidylcholine in human erythrocyte plasma membrane. Differential action of acidic and basic Phospholipase A_2 from *Agkistrodon halysblomhoffi*. J. Biol. Chem., 257: 2908. 1. WHO. Guidelines for the production, control and regulation of snake antivenom immunoglobulins. Geneva: World Health Organization, 2010.
- Fletcher J. E., Rapuano B E., Condrea E., Yang C. C. and Rosenberg P. (1980): Comparison of a relatively toxic Phospholipase A_2 from *Najanigricollis*snake venom with that of a relatively non-toxic Phospholipase A_2 from *Hemachatushaemachatus* venom. II. Pharmacological properties in relationship to enzymatic activity. Biochem. Pharmac. 29: 1565.
- Huang H. C. (1984): Release of slow reactivity substancefrom the guinea pig lung by Phospholipase A_2 of *Viperarusselli* snake venom. Toxicon, 22: 359.
- Banerjee R. N. (1978): Poisonous snakes and their venoms, symptomatology and treatment. In: progress in clinical medicine, second series, Ahuja, MMS (editor). India, Heinemann, 136-179.
- Bhat R. N. (1974): Viperine snake bite in Jammu. Journal of Indian Medical Association. 63, 383-392.
- Ian D. Simpson and Robert L. Norris (2007): Snakes of medical importance in India: Is the concept of the “Big 4” still relevant and useful? Widerness and Environmental Medicine, 18: 2-9.
- Bawaskar H. S., Bawaskar P. H., Punde D. P., Inamdar M. K., Dongare R. B., Bhoite R. R.(2008): Profile of snake bite envenoming in rural Maharashtra, India. JAPI. 56, 88-95.
- Singh A., Biswal N. and Nalini P. (2001): Acute pulmonary edema as a complication of anti-snake venom therapy. Ind. J. Paed. 68: 81-82.
- Corrigan P., Russel F. E. and Wainchal J. (1987): Clinical reactions to antivenin. In: Rosenburg P. (Ed.), Toxins of Animal, Plant and Microbial. Pergamon Press, New York, pp. 457-464.
- Nuno A. P., Ruppelt Pereira B. M., Celio do Nascimento M., Paz Parente J., Mors W. B. (1994): Pharmacological screening of plants recommended by folk medicine as snake venom antidotes; IV. Protection against Jararaca venom by isolated constituents. Planta Medica, 60, 99-100.

- Dravidamani S, Whitaker R, Andrews H. The Irula tribal snake venom extraction co-operative. Part III, Cited on 11/4/08. 160-170.

- Selvanayagam Z. E., Gnavavendhan S. G., Balakrishna K., Bhima Rao R. and Ali S. U. (1995): Survey of medicinal plants with antisnake venom activity in Chengalpattu District, Tamilnadu, India. Fitoterapia. 66, 488-494.
- Abubakar M. S., Sule M. I., Pateh U. U., Abdurahman E. M., Haruna A. K. andJahun B. M. (2000): The in vitro snake detoxifying action of the leaf extract of Guiera senegalensis. Journal of Ethnopharmacology, 69, 253-257.
- Alam M. I., Gomes A. (2003): Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. Journal of Ethnopharmacology, 86, 75-80.
- Jain S. K. (1991) Dictionary of Indian Folk medicine and Ethnobotany. Deelp publications, Paschim Vihar, New Delhi.
- World Health Organization (2001): Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A World Wide Review. Geneva 2001.
- MacDonald Idu(2009): "The plant called medicine" The Guardian March 16, 2009 <http://www.ngrguardiannews.com/focus_record/article03//indexn3_html?pdate=160309&ptitle=The%20plant%20called%20medicine&cpdate=160309>.
- Martz W. (1992): Plants with a reputation against snake bite. Toxicon, 30, 1131-1142.
- Chopra R. N., Nayar S. L., Chopra I. C. (1956): Glossary of Indian medicinal plants. CSIR Publication, New Delhi.
- Kirtikar K. R., Basu B. D. (1975): Indian Medicinal Plants, vols. 1–4. International book Distributors, Dehradun, India, 2793.
- Bocquillon-Limousin H, (1891) Les PlantesAlexiteres de L'Amerique. A. Hennuyer, Paris, 120.
- Selvanayagam Z. E., Gnavavendhan S. G., Balakrishna K. Bhima Rao R. Ali S. U. (1995): Survey of medicinal plants with antisnake venom activity in Chengalpattu District, Tamilnadu, India. Fitoterapia. 66, 488-494.
- Mors WB. (1991): Plants active against snakebite, in Wagner H, Hikino H, Farnsworth NR. (Ed.), Economic and Medicinal Plant Research, vol. 5, Academic Press, London, pp. 352-382.

- Martz W. (1992): Plants with a reputation against snake bite. Toxicon, 30, 1131-1142.
- Houghton P. J., Osibogun I. M.(1993): Flowering plants used against snakebite. Journal of Ethnopharmacology. 39, 1-29.
- Selvanayagam Z. E., Gnavavendhan S. G., Chandrasekkharan P., Balakrishna K., Bhima Rao R. (1994): Plants with antisnake activity- a review on pharmacological and clinical studies. Fitoterapia, 65, 99-111.
- Cherdchu C. and Karlsson E. (1983): Proteolytic-independent cobra neurotoxin inhibiting activity of *Curcuma* sp. (Zingiberceae). South-east Asian J. Trop. Med. Pub. Health. 14: 176-178.
- Homma M., Abe R., Okonogi T., Kosuge T. and Mihima S. (1965): Studies on Habu and Erabu sea snake venoms. Outlines of biological toxicities of two snake venoms and inhibitory actions of tannic acid on them. Jap. J. Bact. 20: 281.
- Okonogi T. and Hattori Z. (1978): Detoxifying effect of persimmon tannin against snake venom. The Snake, 9: 91.
- Mors W. B., Celia doNascimenta M., Ruppelt Pereira B. M., Alvarez Pereira N. (2000): Plant products active against snake bite- the molecular approach. Phytochemistry. 55, 527-642.
- Jayaraman G., Krishnaswamy T., Kumar S. and Yu C. (1999): Binding of nucleotide triphosphates to cardiotoxin analogue II from the Taiwan cobra venom (Najanajaatra). Elucidatio of the structural interaction in the dATP-cardiotoxin analogue ii complex. J. Biol. Chem., 274: 17869-17875.
- Dhananjaya B. L., Nataraju R. R., Raghavendra Gowda C. D., Sharath B. K., Vishwanath B. S. and D'Souza C. J. M. (2006): Anticoagulant effect of *Najanaja*venom 5'nucleotidase: Demonstration through the use of novel specific inhibitor, vanillic acid. Toxicon, 48: 411-421.
- Selvanayagam Z. E., Gnanavendhan S. G., Balakrishna K., Rao R. B., Sivaraman J., Subramanian K., Puri R. and Puri R. K. (1996): Ehretianone, a novel quinonoid xanthenes from Ehretiabuxifolia with antisnake venom activity. Journal of Natural Products, 59: 664-667.

- Alam M. I., Auddy B. and Gomes A. (1994): Isolation purification and partial characterization of Viper venom neutralizing factor from the root extract of Indian medicinal plant Hemidesmus indicus R. British. Toxicon, 32: 1555-1557.
- Wangoda, R.; Watmon, B.; Kisige, M. Snakebite management: experiences from Gulu Regional Hospital, Uganda. East Cent. Afr. J. Surg. 2004, 9(1), 1-5
- Daily monitor. Monday August 31st, 2015. Using nature to get rid of snakes and their venom. https://www.monitor.co.ug/Magazines/HealthLiving/Using-nature-to-get-rid-of-snakes-and-theirvenom/689846-2852038-78tprn/index.html (Accessed 30th August 2019)
- Owuor B.O.; Kisangau, D.P. Kenyan medicinal plants used as antivenin: a comparison of plant usage. Ethnobiol Ethnomed 2006, 2, 7
- Janardhan, B.; Shrikanth, V.M.; Mirajkar, K.K.; More, S.S. In vitro screening and evaluation of antivenom phytochemicals from Azima tetracantha Lam. leaves against Bungarus caeruleus and Vipera russelli. J. Venom. Anim. Toxins incl. Trop. Dis. 2014, 20,1
- Devi, C.M., Bai, M.V.; Lal, A.V.; Umashankar, P.R.; Krishnan, L.K. An improved method for isolation of antiviper venom antibodies from chicken egg yolk. J. Biochem. Biophys. Methods. 2002, 51(2),129–13
- Zolfagharian, H.; Dounighi, N.M. Study on development of Vipera lebetina snake anti-venom in chicken egg yolk for passive immunization. Human Vaccin. Immunother. 2015, 11(11), 2734-2739
- Harrison, R.A.; Hasson, S.S.; Harmsen, M.; Laing, G.D.; Conrath, K., Theakston, R.D. Neutralisation of venom-induced haemorrhage by IgG from camels and llamas immunised with viper venom and also by endogenous, non-IgG components in camelid sera. Toxicon 2006, 47, 364–68.
- Gomes, J.A.S.; Félix-Silva, J.; Fernandes, J.M.; Amaral, J.G.; Lopes, N.P.; Tabosa do Egito, E.S.; et al. Aqueous leaf extract of Jatropha mollissima (Pohl) bail decreases local effects induced by Bothropic venom. BioMed Res. Int. 2016, vol. 2016, Article ID 6101742, 13 pp

(Mr. Nagargoje D.R)
Research Scholar

(Dr. Suresh M Telang)
Research Supervisor